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Chlorella sp. coagulation-flocculation by inducing a modification on the pH broth medium

Daniel dos Reis Fernandes Montes

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Orientador Prof. Doutor Nuno Lapa (Faculdade de Ciências e Tecnologia-UNL)

Co-orientador Prof. Doutor Larry Olson (Arizona State University)

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Abstract

Harvesting algae from the culture medium is a major area of R&D as it is one of the greater obstacles for marketing microalgal oil for biodiesel production at competitive costs when compared to vegetable oils. Former investigations of large scale algae harvesting especially examine the removal of freshwater algal populations from wastewater treatment processes using metal salts like ferric chloride or aluminum sulfate were used. These are effective coagulation-flocculation agents, but, besides of being expensive, their application can be unacceptable if the oil will be used for biodiesel production and the biomass for animal feeding, since the contamination of such products with metals will occur. Indeed, methods based on autoflocculation, biofloculation or even new technologies like electrolytic flotation are promising and could be of very low cost. However, for the moment they require very specific situations and provide significant operating limitations. *Chlorella* characteristics shown to impact on aggregation and flocculation like size, surface functional groups and surface charge were analyzed. Effectiveness of microalgae flocculation by pH manipulation and ionic strength effects were investigated using the Jar-test procedure. A relationship between cell surface at different incubation stages and negative zeta potential for a wide range of pH was checked and it was found that algae removal was not significantly affected by ionic strength.

Keywords:

Chlorella, biodiesel, harvesting, zeta potential, jar-testing, autoflocculation, bioflocculation

Resumo

A colheita de algas do meio de cultura é uma área de investigação e desenvolvimento importante, uma vez que representa um dos maiores obstáculos à comercialização de óleo proveniente de microalgas para a produção de biodiesel a custos competitivos com os óleos vegetais. Investigações prévias focaram-se sobretudo na remoção de algas de estações de tratamento de águas residuais utilizando soluções como a adição de sulfato de alumínio ou cloreto de ferro. Estes agentes coagulantes/floculantes são eficazes, contudo, além de serem dispendiosos economicamente, a sua utilização pode inviabilizar a utilização dos óleos para biodiesel e da biomassa para alimentação animal dado que a biomassa será contaminada. Efectivamente, metodologias baseadas na autofloculação e biofloculação, ou mesmo novas tecnologias como a flotação electrolítica são promissoras e podem ser economicamente vantajosas. No entanto, estas tecnologias ainda requerem condições de operação muito específicas e a sua aplicação é muito limitada. Características da *Chlorella* como o tamanho, grupos funcionais e carga da superfície mostraram estar relacionados e ter impacte na agregação e floculação. Foram testadas diferentes gamas de pH e força iónica num *Jar test* para aferir a sua influência na agregação/floculação. Constatou-se uma relação entre a superfície da parede e a fase de desenvolvimento celular e confirmou-se os valores negativos de potencial zeta para uma vasta gama de pH e uma insignificante influência da força iónica.

Palavras Chave

Chlorella, biodiesel, colheita, potencial zeta, *jar-testing*, autofloculação, biofloculação.

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Abbreviations

APHA- American Public Health Association Water Environment Federation

ASTM- American Society for Testing and Materials

ASU – Arizona State University

AWWA-American Water Works Association

BTL- Biomass-to-Liquid

DAF- Dissolved Air Flotation

DHA- Docosahexaenoic acid (22:6n-3)

EOM- extra cellular Organic Matter

EPA- Eicosapentaenoic acid (20:5n-3)

FTIR- Fourier transformation infrared

GHG- Green house gases

IAF- Induced air flotation

OECD- Organization for Economic Co-operation and Development

PBR- Photobioreactor

PUFA- polyunsaturated fatty acids

TAG- Triacylglycerols

WEF- Water Environment Federation

Table of contents

Table of Figures

List of Tables

Chapter I

1. INTRODUCTION

1.1. RETHINKING BIOFUELS - THE NEW PARADIGM

The finitude of fossil fuels, concerns for energy security and the need to respond to climate change have led to growing worldwide interests in renewable energy sources such as biofuels. The big growth area in recent years has been in the production of liquid biofuels for transport using agricultural crops as feedstock. The bulk of this has taken the form of bioethanol, based on either sugar crops or starchy crops, or biodiesel based on oil crops. Bioethanol and biodiesel are suitable for transportation purposes and they have therefore received substantial attention not only from academia but also in the business scene (Fulton *et al.*, 2004; Chisti 2007). This is because liquid hydrocarbons are well suited for transport uses because of their high energy density and handling convenience. On the other hand, crude oil-based fuels such as gasoline and diesel fuels are mainly used for vehicles traveling over land, kerosene for aircraft, and heavy fuel oils for ships. But as the increasing industrialization and motorization of the world has led to a steep rise in the demand of petroleum-based fuels, it is becoming a key issue for us to produce supplementary fuels from biomass that can be used without requiring substantial modification of existing vehicles or of the fuel distribution infrastructure.

Most recent growth in biofuel production has occurred in the Organization for Economic Co-operation and Development (OECD) countries, predominantly the United States of America and the European Union (EU) countries. An exception is Brazil, which has pioneered the development of an economically competitive national biofuel sector based largely on sugar cane. In the OECD countries, biofuels have been promoted by policies supporting and subsidizing production and consumption; such policies are now being introduced in a number

of developing countries. The main drivers behind OECD country policies have been the objectives of energy security and climate-change mitigation through reduced greenhouse gas emissions combined with a desire to support agriculture and promote rural development. These concerns are not diminishing; indeed, climate change and future energy security continue to move higher up the international policy agenda.

However, political and public support for biofuels has been undermined due to environmental and food security concerns as well as by recent reports questioning the rationale that biofuels substantially reduce carbon emissions. The diversion of food crops or croplands to produce biofuels has been blamed for global food shortages and associated increasing costs of staple food crops such as maize and rice (James *et al.,* 2008; Josserand, 2008; Rahman *et al*., 2008).

Also, recent research suggests that certain biofuel production pathways may lead to net positive GHG emissions or substantial carbon debts. That's what is happening with the conversion of carbon-rich peatland to oil palm plantations in Southeast Asia (Scharlemann and Laurance, 2008). Nevertheless, some policy makers and scientists remain optimistic that with the development of 'next generation' biofuels such as algae biodiesel and cellulosic ethanol there are real opportunities for using biomass to meet some of our energy needs (Ragauskas *et al*., 2006).

1.2. PROGRESS AND RECENT TRENDS IN BIODIESEL FEEDSTOCKS

Among liquid biofuels, biodiesel specially derived from vegetable oils is gaining market share as diesel fuel all over the world. Global biodiesel production increased from 1.8 million tons in 2002 to 11.8 million tons in 2008. Despite of its relatively high production costs, the limited availability of some raw materials used in its production continue to limit its commercial

application. The major economic factor to consider with respect to the input costs of biodiesel production is the feedstock, which is about 80% of the total operating cost (Armbruster and Coyle, 2006).

A variety of feedstock can be used to produce biodiesel. In Figure 1.1 it is represented an overview for raw material consumption for biodiesel production in the past and recent future predictions. Virgin vegetable oils feedstock are by far the most used oils and they're expected to keep like this in the next few years. Rapeseed, soybean and palm oils are the most commonly used vegetable oils. Waste vegetable oil, animal fats including tallow, lard, and yellow grease and other vegetable oils such as mustard, sunflower, castor, jatropha and even algae are being used and show promise but they are not expected to have a significant impact in the near future.

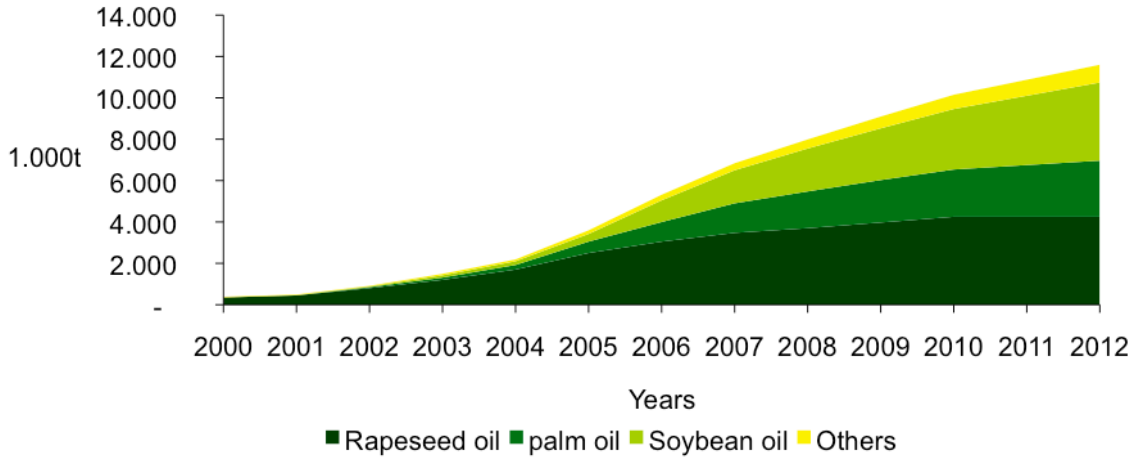

Figure 1.1: Vegetable oil consumption for biodiesel production in the past and recent future predictions (Adapted from Thurmond, 2008)

1.3. MICROALGAE AS BIOFUELS FEEDSTOCK AND CO_2 BIO-SEQUESTRATION STRATEGY

Perhaps the largest potential for carbon neutral energy comes from the conversion of sunlight into high-value photosynthetic microorganisms. Certain algae (eukaryotes) and cyanobacteria (prokaryotes) have high lipid contents that can be extracted and converted to biodiesel. Apart from high efficiency production of oil for biodiesel, microalgae are also well suited for the production of feedstocks for other biofuels. The development of the technologies for high efficiency algal production is also applicable to biohydrogen, biogas, bioethanol and biomass-to-liquid (BTL) approaches.

In **Table 1.1**, microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel.

Table 1.1: Comparison of some sources of biodiesel (Adapted from Cisthi, 2007)

Crop	Oil yield (L/ha)	Land area needed (Mha)[a]	% of existing US cropping area [a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
jatropha	1892	140	77
Coconut	2689	99	54
Oil Palm	5950	45	24
Microalgae[b]	136.900	2	1,1
Microalgae[c]	58.700	4,5	2,5

a) for meeting 50% of all transportation fuel needs of the United States
b) 70% oil (by wt) in biomass
c) 30% oil (by wt) in biomass

Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Microalgae commonly double their biomass within 24 h. Biomass doubling times during exponential growth are commonly as short as 3.5 h.

Based upon the photosynthetic efficiency and growth potential of algae, theoretical calculations indicate that annual oil production of >30 000 l or about 200 barrels of algal oil per hectare of land may be achievable in mass culture of oleaginous algae, which is 100-fold greater than that of soybean, a major feedstock currently being used for biodiesel in the USA. While the 'algae-for-fuel' concept has been explored in the USA and some other countries, with interest and funding growing and waning according to the fluctuations of the world petroleum oil market over the past few decades, no efforts in algae-based biofuel production have proceeded beyond rather small laboratory or field testing stages. The lipid yields obtained from algal mass culture efforts performed to date fall short of the theoretical maximum (at least 10–20 times lower), and have historically made algal oil production technology prohibitively expensive (Hu *et al*., 2006; Sheehan *et al*., 1998).

Concerning the CO_2 sequestration, there is a worldwide awareness about global warming as a result from the rising levels of different greenhouse gases released from the burning of fossil fuels. Different methods have been suggested as to how CO_2 could be sequestered or immobilized through different processes and subjected for long-term storage to avoid release into the atmosphere. Physical sequestration of atmospheric CO_2 is often considered challenging, as it is technically difficult to separate CO_2 from other atmospheric gases. Photosynthetic organisms have however fine-tuned this process over millions of years and of course are well adapted to capture CO_2 and store it as biomass. In this respect, the idea of biological sequestering by growing algae and take advantage of their photosynthetic machinery of capturing carbon dioxide has been suggested by many as an alternative method of reducing the amount of CO_2 released in the atmosphere (Benemann, 1997; Hughes and Benemann, 1997; Chisti, 2007; Kadam, 1994, 1995 and 1997). To use micro-algae to fix CO_2

released from power plants via the exhaust gas and thereby mitigate the amount of carbon released into the atmosphere is an attractive idea. However, there are several major challenges before this idea becomes practical. Strains that grow well at CO_2 concentrations of 5-10% show drastic decreases in their growth rate above 20% (Watanabe *et al*., 1992). An important task therefore has been to identify strains that can cope with very high CO_2 concentrations and also have high growth rates. Screening has yielded strains that grow well in CO_2 concentrations between 30% and 70% saturation (Hanagata *et al*., 1992; Iwasaki *et al*., 1996). Also, results by Olaizola (2003) indicate that by controlling the pH changes in the culture and releasing CO_2 to the algae on demand, growth could be sustained even at 100% CO_2. Another important property that would need to be optimized is the ability of algae strains to have high thermal stability. It has been suggested that the hot flue gases introduced in the algal cell cultures may influence the temperature (Ono and Cuello, 2007).

1.4. PROJECT GOALS AND TASKS

Among the various algae species that are cultivated in the ASU algae laboratory, green algae *Chlorella* was decided by the Lab as the most interesting. The genus *Chlorella* is a large segment of the *Chlorophyceae* with many diverse species. Some of these species are of interest to the dietary supplement or nutraceutical industry, because of their production of polyunsaturated fatty acids (PUFA), antioxidants (like β-carotene), and other beneficial products. These species are also of interest to the growing research area of algal biodiesel. Here, the triglycerides and other fatty acids molecules produced by *Chlorella* are a potential feedstock for biodiesel. These two areas provide the motivation for understanding the metabolic pathways for lipid production and the conditions under which lipid production can be maximized in *Chlorella*.

Harvesting algae from the culture medium is a major area of R&D as it is one of the greater obstacles for marketing microalgal oil for biodiesel production at competitive costs when compared to vegetable oils. Recovery of the biomass from the growth medium is one of the major problems of the productive process since it represents 20–30% of the total cost of algal biomass production (Molina *et al*., 2002).

Former investigations of large scale algae harvesting especially examine the removal of freshwater algal populations from wastewater treatment processes using different technologies (Clasen *et al.,* 2000; Henderson *et al*., 2006; Phoochinda *et al.,* 2003; Pieterse *et al.,* 1997). Usually metal salts like ferric chloride or aluminum sulfate were used. These are effective coagulation-flocculation agents, but, besides of being expensive, their application can be unacceptable if the oil will be used for biodiesel production and the biomass for animal feeding, since the contamination of such products with metals will occur (Benneman, 1996).

Under this context, the main aims of this research were as follows:

- Algae culturing and harvesting literature review and technology state-of-art;
- Obtain a better understanding of the cause and mechanisms of algal aggregation and flocculation, focused in understanding the pH effect, different culture conditions and different life stages in *Chlorella* sp.;
- Inducing coagulation-flocculation of microalgae with pH adjustement, without using chemical coagulation-flocculation agents;
- Determination of Zeta Potential of *Chlorella* sp. in different life stages and testing the coagulation-flocculation of *Chlorella* sp. through the adjustment and control of pH medium by using Jar-tests.

Chapter II

2. LITERATURE REVIEW

2.1. ALGAE CULTIVATION FOR BIOFUELS

Exploitation of micro-algae for bioenergy generation (biodiesel, biomethane, biohydrogen and bioethanol), or combined applications for biofuels production and CO_2-mitigation are still under research, but mankind for millenniums has used micro-algae. They find uses as food and as live feed in aquaculture for production of bivalve molluscs, for juvenile stages of abalone, crustaceans and some fish species and for zooplankton used in aquaculture food chains. Therapeutic supplements from micro-algae comprise an important market in which compounds such as β-carotene, astaxanthin, polyunsaturated fatty acid (PUFA) such as DHA and EPA and polysaccharides such as β-glucan dominate (Spolaore *et al*., 2006).

2.2. ALGAE SPECIES AND OIL CONTENT

Oxygenic photosynthetic microalgae and cyanobacteria represent an extremely diverse, yet highly specialized group of microorganisms that live in diverse ecological *habitats* such as freshwater, brackish, marine and hyper-saline, with a range of temperatures and pH, and unique nutrient availabilities (Falkowski and Raven, 1997). With over 40 000 species already identified and with many more waiting to be identified, algae are classified in multiple major groupings as follows: cyanobacteria (*Cyanophyceae*), green algae (*Chlorophyceae*), diatoms (*Bacillariophyceae*), yellow-green algae (*Xanthophyceae*), golden algae (*Chrysophyceae*), red algae (*Rhodophyceae*), brown algae (*Phaeophyceae*), dinoflagellates (*Dinophyceae*) and 'pico-plankton' (*Prasinophyceae* and *Eustigmatophyceae*).

Of the strains examined, green algae represent the largest taxonomic group from which oleaginous candidates have been identified. This may not be because green algae naturally

contain considerably more lipids than other algal *taxa*, but rather because many green algae are ubiquitous in diverse natural habitats, can easily be isolated, and generally grow faster than species from other taxonomic groups under laboratory conditions (Hu *et al*., 2008).

As many algal species have been found to grow rapidly and produce substantial amounts of TAG or oil, and are thus referred to as oleaginous algae, it has long been postulated that algae could be employed as a cell factories to produce oils and other lipids for biofuels and other biomaterials (Benemann *et al*., 1982; Borowitzka, 1988; Sheehan *et al*., 1998). Oil content in microalgae can exceed 70% by weight of dry biomass (Chisti 2007; Spolaore *et al*., 2006). Oil levels of 20–50% are quite common (Table 2.1).

Table 2.1: Oil content of some microalgae (adapted from Chisti, 2007)

Microalga	Oil Content (% dry wt)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

2.2.1. Fatty acid composition of algae

Depending on species, microalgae produce many different kinds of lipids and complex oils. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Algal lipids are typically composed of glycerol, sugars, or bases esterified to fatty acids having carbon

numbers in the range of C12-C22. They may be unsaturated or saturated. Eukaryotic algae predominantly contain saturated and unsaturated fatty acids, triglycerides are the most common storage lipids constituting up to 80% of the total lipids fraction (Chisti, 2007). Nutritional and environmental factors can affect both the relative proportions of fatty acids as well as the total amount (Gouveia *et al*., 2008). Oleaginous algae can be found among diverse taxonomic groups, and the total lipid content may vary noticeably among individual species or strains within and between taxonomic groups.

Table 2.2: Main fatty acids present in different oil extracts (Adapted from Gouveia and Oliveira., 2008)

Fatty acid	*Chlorella sp.*	*Scenedesmus obliquus*	*Dunaliella tertiolecta*	*Nannochloropsis sp.*	*Neochloris oleabundans*
14:0	3.07	1.48	0.47	7.16	0.43
16:0	25.07	21.78	17.70	23.35	19.35
16:1	5.25	5.95	0.88	26.87	1.85
16:2	n.d.	3.96	3.03	0.39	1.74
16:3	1.27	0.68	1.24	0.48	0.96
16:4	4.06	0.43	10.56	n.d.	7.24
18:0	0.63	0.45	n.d.	0.45	0.98
18:1	12.64	17.93	4.87	13.20	20.29
18:2	7.19	21.74	12.37	1.21	12.99
18:3	19.05	3.76	30.19	n.d.	17.43
18:4	n.d.	0.21	n.d.	n.d.	2.10
20:0	0.09	n.d.	n.d.	n.d.	n.d.
20:1	0.93	n.d.	n.d.	n.d.	n.d.
20:2	n.d.	n.d.	n.d.	n.d.	n.d.
20:3	0.83	n.d.	n.d.	n.d.	n.d.
20:4	0.23	n.d.	n.d.	2.74	n.d.
20:5	0.46	n.d.	n.d.	14.31	n.d.
Saturated	28.56	23.71	18.17	30.96	20.76
Unsaturated	51.91	54.66	63.14	59.20	64.60

All microalgal lipids are mainly composed of unsaturated fatty acids (50–65%) and a significant percentage of palmitic acid (C16:0) was also present (17–40%). Among the unsaturated fatty acids, special attention should be taken to the linolenic (C18:3) and polyunsaturated (4 double

bonds) acids, due to the EN 14214 that species a limit of 12% and 1%, respectively, for a quality biodiesel. However, the analyzed microalgae oils may be used for good quality biodiesel if associated with other oils or without restrictions as raw material for other biofuels production processes (Gouveia and Oliveira, 2008).

2.2.2. Maximizing oil content and lipid metabolism in *Chlorella*

Fatty acids synthesis in *Chlorella*, as in all *Chlorophyta*, is oxygen dependent and produces mostly fatty acids of chain lengths from 16 to 22 carbons. Factors such as nutrient concentration, salinity, light intensity, and temperature influence the lipid content and type (for instance polar and non polar lipids) present in cells.

In algal cells, neutral lipids (triglycerides) are used as a form of carbon and energy storage, while phospholipids and glycolipids are in the class of polar lipids, whose function includes forming cell and chloroplast membranes (Guckert and Cooksey, 1990). Although polar lipids can be made into biodiesel, traditional feedstocks are triglycerides. Therefore, non-polar lipids are the more desirable algal product.

Otsuka and Morimura (1966) synchronously have grown cultures of *Chlorella ellipsodea* to demonstrate the change in the fatty acid composition over the stages of cell growth. The resulting fatty acid profile, organized by growth stage, showed a change in the relative distribution of polar and non-polar fatty acids at successive stages in the cell growth. *Chlorella ellipsodea* had a spike in the polar fatty acid content in early growth phase and a spike in non-polar fatty acids closer to cell division. The accumulation of polar lipids happens during growth stages, where the accumulation on non-polar fatty acids is a buildup of energy reserves prior to the cell division.

It was found that oleic acid (C18:1) and palmitic acid (C16:0) are the most abundant fatty acids at most growth stages. C18:1 was consumed during cell division regardless of whether the cells

were under light or dark conditions, suggesting that C18:1 in triglycerids is a major source of energy during cell division or that is important for various synthesis process that may be a part of cell division. As is characteristic of green algae, 80% of the fatty acids in *Chlorella ellipsodea* are unsaturated fatty acids, and palmitic acid is the only saturated fatty acid found in significant quantity in the cell (Otsuaka and Morimura, 1966). This has important implications for nutraceutical use, which may focus on the high Poly Unsatured Fatty Acids (PUFA) production, and for beneficial cold weather properties of biodiesel made from this oil.

Guckert and Cooksey (1990) performed experiments that showed how the fatty acid profile of *Chlorella* sp. changes with the pH. The intent of the experiments was to show how the fatty acids profile changed with nitrogen deficiency and silicate can increase neutral lipid in diatoms. Instead, it was found that neutral lipids accumulation happened before any nitrogen deficiency could occur. Lipid accumulation generally happens because the cell growth cycle is inhibited at some points and cell division is delayed. During this time, the cell continues to make and store lipids in anticipation of division, thus the neutral lipid content will increase.

The experiments showed that cultures submitted to higher pH values exhibited slowed growth and generally had a lower cell concentration. The high pH cultured cells were also the biggest producers of triglyceride (Guckert and Cooksey, 1990).

2.2.3. Algae Culturing Conditions

The optimization of strain-specific cultivation conditions is of confronting complexity, with many interrelated factors that can each be limiting. These include temperature, mixing, fluid dynamics and hydrodynamic stress, gas bubble size and distribution, gas exchange, mass transfer, light cycle and intensity, water quality, pH, salinity, mineral and carbon regulation/bioavailability, cell fragility, cell density and growth inhibition (Schenk *et al*., 2008).

The vast majority of research into algal growth and nutrient relations has traditionally been devoted to algae in its natural environments and the role of algal growth within ecosystems. In contrast, algal production systems require maximum biomass growth to very high cell densities. A better understanding of the physical principles and bioreactor design has increased the current achievable cell density. Optimal media formulation is also critical to ensure sufficient and stable supply of nutrients to attain maximal growth acceleration and cell density, and ultimately to produce biofuels at higher efficiencies (Dayananda *et al*., 2005). Algal production can also be a multi-phased process with each step having independent optimal conditions such as nitrogen limitation in oil production (Singh *et al*., 1992). Batch feeding of heterotrophic algal cultures (Li *et al*., 2007) and CO_2 enrichment of photoautotrophic algal cultures can significantly increase biomass, and optimization of mineral nutrients can increase culture productivity. Nitrogen and phosphorous are generally early targets in mineral optimization of media formulations, but other minerals are also vitally important for support of the structural and metabolic biochemistry of the cell. Mineral ions also have significant impact upon areas such as osmotic regulation and osmotic adaptive capacity, and molecular configuration of photosynthetic complexes (Merchant *et al*., 2006).

Certain algal strains when grown with yeast extract, grow at twice the rate and to twice the cell density. The strains that show improved growth are clearly able to assimilate the dissolved organic molecules, and consequently in some circumstances wastewater can therefore be considered a resource. The increasing scarcity of fresh water resources in many countries also makes this recycling of wastewater attractive and different aspects of wastewater usage continue to be investigated (Sawayama *et al*., 1992). Maintenance of an acceptable pH range throughout culturing is of utmost importance as it impacts all aspects of media biochemistry. Both, ionic absorption from the media and the metabolic biochemistry of the cell, exert

significant pressure upon pH and in high performance cultures their effect is powerful enough to overcome the neutralizing capacity of exogenous buffering agents. Currently microinjection of strong acids and alkalis, metabolic balancing in heterotrophic cultures and regulated CO_2 dissolution in both photoautotrophic and heterotrophic cultures, are the most practical and economical strategies for pH control (Stumm and Morgan, 1996).

2.2.4. Microalgal cultivation systems

Conventional open pond algal production systems and even some closed algal bioreactors have already achieved economic viability in the production of high value products such as astaxanthin and nutraceuticals. However, economic margins are much smaller for biofuel production systems as their market value is much lower. Optimized biomass production is therefore central to economic biofuels production (Li *et al*., 2007; Schenk *et al*., 2008) and this in turn requires careful optimization of cultivation systems.

The design of large-scale culture systems have to consider many factors, including light intensity, temperature, biology of the algae, nature of the product, mixing, aeration, source of carbon dioxide and sterilization, etc. (Borowitzka, 1999). Although microalgae are considered to be relatively efficient for capturing solar energy for the production of organic compounds via photosynthetic process, the photosynthetic efficiency of microalgae for the conversion of solar energy is typically below 20% (Li *et al*. 2007). On the other hand, increasing the density of cultures decreases photon availability to individual cells, which reduces specific growth rate of cells. Therefore, the poor penetration of light could be the most significant limiting factor in microalgal cultivation.

There are two primary types of cultivation systems, open ponds (Figure 2.1.) with moderate surface to volume ratios and photobioreactors (Figure 2.2.) with high surface to volume ratios (Weissman *et al*., 1988).

The most common design of open ponds (Tredici and Materassi, 1992) is raceway ponds, in which algal cultures are mixed in a turbulent flow sustained by a paddle wheel. At least two types of open raceway ponds have been used commercially. The first is raceway ponds lined by concrete, and the second is a shallow earthen tunnel lined with polyvinyl chloride or other durable plastic.

Figure 2.1: Aerial photographs of different open pond designs. a) Extensive unstirred ponds located at Hutt Lagoon, Western Australia used for culturing *Dunaliella salina* (pond area > 200 ha); b) Inclined ponds used for culture of *Chlorella* and *Scenedesmus* at Trebon, Czech Republic; c) Central pivot pond and culture for *Chlorella* in Taipei, Taiwan; d) Raceway ponds at the Earthrise *Spirulina* plant at Calipatric, California, USA. (Courtesy of M. A. Borowitzka and A. Belay)

While open ponds require low cost to cultivate algal biomass, photo-bioreactors provide other advantages (Rosello *et al.*, 2007) such as large surface/volume ratios, ability to prevent contamination, capacity to achieve high density of biomass and high biomass productivity, and therefore, high CO_2 fixation rate (Table 2.3). The tubular photobioreactor, one of the most popular configurations of photobioreactors, includes an array of straight transparent tubes, which are used to capture sunlight. Relatively small tube diameter, generally 0.1 m or less, is

necessary for ensuring high biomass productivity. In a typical arrangement, the solar tubes are placed parallel to each other horizontally. A variety of other photobioreactors have also been studied.

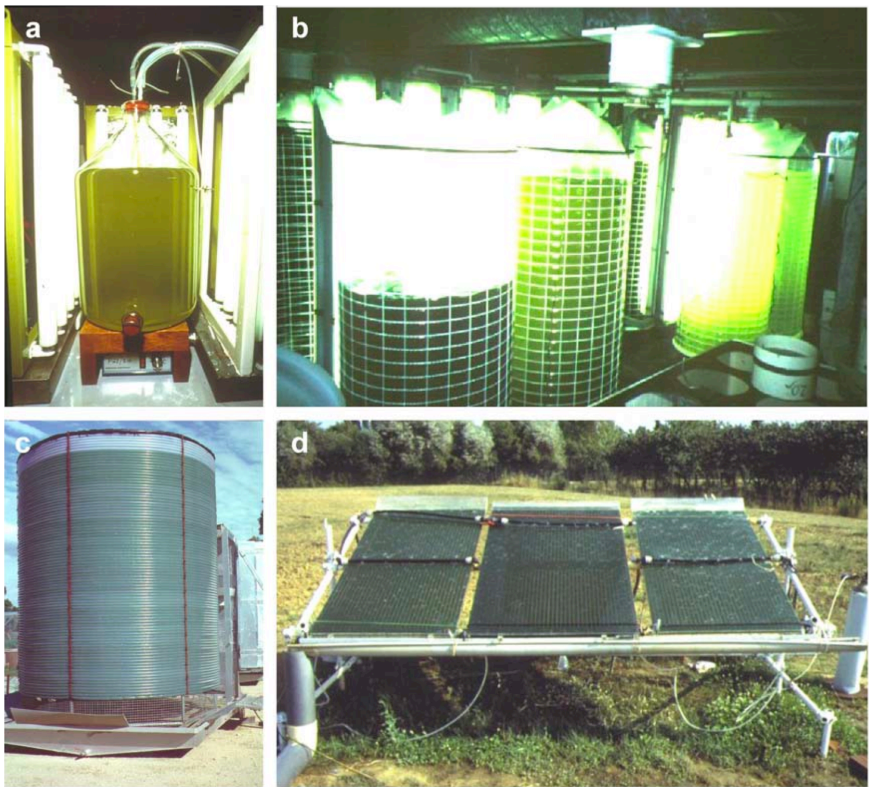

Figure 2.2: Different closed photobioreactor designs (a) a stirred and aerated carbon, (b) bag driven tubular photobioreactor, (c) 1000 L outdoor airlift photobioreactor (Biocoil) and (d) inclined plate photobioreactor (a - c courtesy of M. A. Borowitzka and d courtesy of A. Richmond).

For instance, a flat-plate photobioreactor (Hu *et al*., 1996) has been tested for cultivation of high-CO_2 tolerant unicellular green alga *Chlorococcum littorale*. Bubble sparged and airlift photobioreactors (Kaewpintong *et al*., 2007) are also common choices for microalgae cultivation. Analyses of designed system showed that CO_2 mitigation costs depend closely on the productivity of algae and the intensity of solar radiation (Kadam, 1997). The main problem associated with the use of photobioreactors is the intensive capital cost (Terry and Raymond, 1985), which is expected to be overcome by technology development.

Table 2.3: Comparison between raceway ponds and tubular photobioreactors of microalgal cultivation (Adapted from Wang *et al*., 2008)

Parameter	Raceway ponds	Tubular Photobioreactores
Light efficiency	Fairly good	Excellent
Temperature control	None	Excellent
Gas transfer	Poor	Low-high
Produced oxygen	Low	High
Hydrodynamic stress on algae	Difficult	Easy
Species control	None	Achievable
Sterility	Low	High
Cost to scale up	Low	High
Volumetric productivity	High	Low

2.3. HARVESTING AND DEWATERING PROCESS

The literature on microalgae harvesting is extensive, starting with the first studies of algal mass cultures. However, none are universally satisfactory on both an economic and performance basis, and relatively little or no experience exists with low cost harvesting for biofuels production. Indeed, most experience is with harvesting microalgae in wastewater treatment plants, where there was little control over cultivation conditions or algal species (Benneman, 1996). For the particular algae that we are focusing on, centrifugation has been the only commercial technology used and *Chlorella* manufacturing plants typically have a battery of centrifuges, which are characterized by major capital investment and high operating cost factor.

Harvesting of biomass requires one or more solid–liquid separation steps. This is considered to be an expensive and problematic part of industrial production of microalgal biomass. Recovery of the biomass from the broth has been claimed to contribute 20–30% to the total

cost of producing the biomass (Gudin and Therpenier, 1986). Especially due to the low cell density achievable with microalgal cultures, which is typically in the range of 0.3–0.5 g dry cell weight per liter and with exceptional cases reaching 5 g dry cell weight per liter (Molina *et al*., 2002). On the other hand, the optimal material for industrial conversion is a cell sludge containing at least 300–400 g dry cell weight per liter, implying that the effluent algal suspension needs to be concentrated 100–1.000 times (Ginzburg, 1993), which could be an energy intensive process.

2.4. MICROALGAE CHARACTERISTICS WITH RESPECT TO THEIR SEPARABILITY FROM AQUEOUS SUSPENSIONS

The algae characteristics that have showed more impact on wastewater treatment were morphology, motility, surface charge, cell density and the extracellular organic matter (EOM) composition and concentration (Henderson *et al*., 2006).

Cell morphology, including shape, size, and appendages, is used for classification purposes but only once identified to *phylum* level as key cell shapes exist in all *phyla*. Single spherical cells of less than 5 µm are common in green algae like *Chlorella* and *Scenedesmus quadriculata* (Table 2.4).

Table 2.4: Algae characterization form a water treatment perspective (Adapted from Henderson *et al*., 2006)

Algae	Size (µm)	Colonial	Shape	Density ($kg.m^{-3}$)	Zeta Potential (mV)	Test conditions (pH; test water)
Chlorella vulgaris	5,3	Single cell	Sphere	1070	-10	7; Distilled water
					-19,8 (log growth)	6,5; Reservoir water
					-17,4 (stationary growth)	

***Chlorella* sp.**	3,5	Single cell	Sphere	1070	-10	4-8; distilled water
					-14,9 to -19,8	5,41-7,08 ; Synthetic test water
Scenedesmus quadriculata	13,1	Row(s) of 4-16 cells with spinal appendages	Ellipsoidal		-25 to -35	7-10; Distilled water
Nitzchia linearis	35	Single cell	Needle	1100	-30 to -40	7,5-7,7

Cell density is observed to be around 1070 $kg.m^{-3}$ to for green algae *Chlorella* (Edzwald and Wingler, 1990), although densities as low as 1020 $kg.m^{-3}$ have been quoted for a typical algal entity (Pieterse and Cloot, 1997).

Both the electric repulsion interactions between algal cells and cell interactions with the surrounding water contribute to the stability of the algal suspension (Tenny *et al*., 1968). The intensity of that charge is a function of algal species, ionic strength of medium, pH and other environmental conditions (lves, 1959). The sources of the algal surface electric charge are: ionization of ionogenic functional groups at the algal cell wall (Golueke and Oswald, 1965) and selective adsorption of ions from the culture medium (Beckett and Lee, 1990; Ulberg and Morochko, 1999).

The electric state of a surface depends on the spatial distribution of free charges (ions) in its neighborhood (Stumm and Morgan, 1996) and it is idealized as an electrochemical double layer. One layer is described as a fixed charge attached to a particle surface and is called the Stern layer. The other is called Gouy layer or diffuse layer that contains an excess of counter ions (ions of opposite sign to the fixed charge) and a deficit of co-ions (of the same sign as the fixed charge). Neither the potential at the surface nor the potential at the border of Stern and diffuse layer can be directly measured. Instead, the zeta potential, the potential measured at

the shear plane (that separates the solid surface and the mobile liquid), is the one generally used and is obtained by simple electrokinetic methods.

Algae suspension surface charge is species but not *phyla* dependent, although all algal cells have a negative zeta potential at natural water pH (Table 2.4). The zeta potential of an algal cell is typically electronegative for pH 4–10, ranging from -10 mV for *Chlorella* to -35 mV for *Scenedesmus*. In general, an isoelectric point of around pH 3–4 is determined for all algal species (Stumm and Morgan, 1996; Liu *et al*., 1999; Clasen *et al*., 2000; Phoochinda and White, 2003). The stage of life cycle can also influence zeta potential. To illustrate, the diatom *Nitzschia* had a zeta potential of -30 mV at the initial growth phase, -35 mV in the log growth phase and -28 mV in the stationary phase (Konno, 1993) and the surface charge of *Chlorella* also became less negative on transition from log growth phase to stationary phase, measured as -1.6 to -1.4 $mmVs^{-1}cm^{-1}$ (-19.8 to -17.4 mV) (Edzwald and Wingler, 1990). It has been postulated that this phenomenon is due to variations in quantity and composition of EOM attached to the cell surface (Bernhardt *et al*., 1994). This implies that it is the organic matter present that controls the surface charge as opposed to the cell surface itself.

2.5. CURRENTLY USED AND ALTERNATIVE HARVESTING METHODS

Microalgae harvesting processes used in large-scale commercial installations are generally proprietary. Relatively little information is available, with the exception of wastewater treatment facilities. Even with these there is a lack of specific data. Thus, this discussion is general in nature. As the commercial plants produce algae worth many thousands of dollars

per ton, they are not constrained by the economics of fuel or commodities production and their harvesting technologies would not likely be useful in this context.

2.5.1. Centrifugation

Centrifugation is a well established process in microbial biotechnology and several equipment manufacturers sell industrial units. Centrifugal recovery of the biomass is feasible for high-value microalgae products. It was used for algae harvesting since the early studies of algal mass culture of *Chlorella* (Golueke and Oswald, 1965). These authors studied centrifugation of sewage grown microalgae with a field-scale nozzle centrifuge, which could be operated with various disc angles in the rotor. From a process perspective, as the algal media can be recycled, the harvest efficiency is a secondary consideration to the total amount of algae recovered per unit time, which is also proportional to unit power requirement (the major operating cost).

Many other authors have tested various centrifuges for algae harvesting, but the only detailed studies where by Mohn (1980). He tested various centrifuges - all had some drawbacks and high costs associated. As above, the nozzle disc type centrifuge with intermittent discharge was concluded to be the best option. One requirement is that all feed be carefully pre-screened to remove larger materials, debris etc., which can clog the nozzle, abrade the centrifuge, etc. He also estimated relative capital costs and operating power costs for various harvesting methods with centrifugation being the most expensive on both accounts. From this data, it would require (at 333 mg/l) 3,000 kWh/t of microalgae, similarly of what was estimated by Golueke and Oswald (1965). This is, clearly, excessive for any CO_2 mitigation project, as energy inputs (and, thus, CO_2 emissions) would exceed the fuel value of the recovered algal biomass. In conclusion, centrifugation is clearly impractical for primary

harvesting method. However, it can be considered as a secondary harvest method, to concentrate an initial slurry, from a few (1 - 5%) solids to produce an algal slurry or paste (15 to 20% solids), such as would be required for lipid extraction or otherwise processing the biomass.

2.5.2. Flocculation

Various methods of flocculation can be used to aggregate the microalgal cells to increase the effective particle size and hence, ease sedimentation, centrifugal recovery and filtration (Elmaleh *et al*., 1991). Microalgal cells carry a negative charge that prevents aggregation of cells in suspension. The surface charge can be neutralized or reduced by adding flocculants such as multivalent cations and cationic polymers to the broth. Ideally, the flocculants used should be inexpensive, nontoxic, and effective in low concentration. In addition, the flocculant should be selected so that further downstream processing is not adversely affected by its use.

2.5.2.1. Multivalent metal salts

Multivalent metal salts are effective flocculants or coagulants. The commonly used salts include ferric chloride ($FeCl_3$), aluminum sulfate ($Al_2(SO_4)_3$, alum) and ferric sulfate ($Fe_2(SO_4)_3$). The efficiency of electrolytes to induce coagulation is measured by the critical coagulation concentration, or the concentration required causing rapid coagulation. Coagulation efficiency of metal ions increases with increasing ionic charge. Multivalent metal salts such as alum have been widely used to flocculate algal biomass in wastewater treatment processes (Benemann *et al*., 1980; Moraine *et al*., 1980; Koopman and Lincoln, 1983). The major problem was the very high amount of chemicals required. Lime flocculation was optimal when 120 mg $Ca(OH)_2$ were combined with 40 mg $FeSO_4$. Dosages over 100 mg/l of alum were

required to achieve a 90% clarification of the waste pond effluent. Recovery of the alum by acidification was studied, but proved to be relatively unsuccessful. Tests for alum flocculation of algae from the Auckland, N.Z., oxidation ponds were feasible, though expensive. However, cost chemical flocculation was estimated at roughly 40% lower than those of centrifugation (Edzwald, 1993).

Alum is an effective flocculant for *Scenedesmus* and *Chlorella* (Golueke and Oswald, 1965). However, flocculation by these metal salts may be unacceptable if biomass is to be used in certain aquaculture and other applications (Benneman, 1996). Golueke and Oswald (1965), who studied lime and alum flocculation, found, as expected, removal efficiencies dependent on pH and dosage, as well as the algal culture themselves. Also, extracellular products of microalgae can interfere in the flocculation process (Bernhardt *et al*., 1985; Hoyer *et al.*, 1985).

2.5.2.2. Polyelectrolytes

An alternative to using metal salts is the use of cationic polymers (polyelectrolytes) (Tenney *et al.*, 1969). In addition to reducing or neutralizing the surface charge on cells, the polymer flocculants can bring particles together by physically linking one or more particles through a process called bridging. Tenney *et al*. (1969) and Tilton *et al*. (1972) have demonstrated that the bridging mechanism also applies to flocculation of algal cells.

Cationic polymers doses of between 1 and 10 $mg.ml^{-1}$ can induce flocculation of freshwater algae; however, a high salinity of the marine environment can inhibit flocculation by polyelectrolytes (Bilanovic *et al*., 1988). Effective flocculation with polyelectrolytes has been reported at salinity of less than 5 $kg.m^{-3}$. At high ionic strengths, cationic polymers tend to fold tightly and fail to bridge microalgal cells.

In all cases, the flocculation was reduced by increasing ionic strength of the cell slurry. The flocculation effectiveness of polyelectrolytes depends on many factors, including the molecular mass of the polymer, the charge density on the molecule, the dose used, the concentration of the biomass, the ionic strength and pH of the broth, and the extent of mixing the fluid. Generally, high molecular weight polyelectrolytes are better bridging agents. Similarly, a high charge density tends to unfold the polymer molecule, improving its bridging performance and the ability to neutralize the surface change on cells. A high cell concentration in the broth helps flocculation, because the cell–cell encounters are more frequent in concentrated suspensions. A certain low level of mixing is useful as it helps bring the cells together; however, excessive shear forces can disrupt flocs (Chisti, 1999).

Polymeric flocculants have been used extensively for recovering microalgal biomass. However, in comparison with salts such as aluminum sulfate, cationic polyelectrolytes may be less effective (Pushparaj *et al*., 1993). Studies with *Chlorella ellipsoidia* at biomass concentration of 0.05–3 $kg.m^{-3}$ and polymer concentration of $1x10^{-5}$ to 1 $kg.m^{-3}$ have shown an absence of flocculation at polymer concentration up to 0.2 $kg.m^{-3}$ (Tilton *et al*., 1972). Cationic polyethyleneimine is an effective flocculant for *Chlorella*. In studies with *Chlorella*, the amount of polymer required to initiate flocculation decreased as the molar mass of the polymer increased. However, further increases in molar mass did not improve flocculation efficiency. Changes in pH over the range of pH 4–7, did not affect flocculation behavior (Tilton *et al*., 1972). Polyvalent organic polymers have been claimed as effective flocculants for *Scenedesmus* and *Chlorella* (Golueke and Oswald, 1965).

2.5.2.3. Natural Settling

Natural settling is the spontaneous gravity sedimentation of algal cells after removal from the cultivation system. As most algal cells, or even colonies, are rather small, they do not exhibit sufficiently high settling rates to allow their practical (low cost) recovery by simple gravity sedimentation. From the Stokes law, only relatively large (>100 nm diameter) colonies or aggregates of cells (flocs) will allow gravity sedimentation, over a reasonable time period, to produce a biomass concentrate (typically 1.5 to 4%). The term "natural settling" encompasses all phenomena by which algae, after removal from the cultivation system exhibit acceptable settling rates, without addition of flocculating chemicals. Acceptable settling rates are herein defined as at a minimum of 10 cm/hr (Benemann *et al*., 1982), but preferably several fold higher. If settling rates are high enough (> 1 m/h) continuous processes (clarifiers) could be used. At the lower settling ranges, the preferred process would be batch settling using deep (e.g. 2-3 m) ponds. Natural settling in this context can be either positive or negative (e.g. natural flotation). Natural flotation could be achieved by means of gas vacuoles (blue-green algae) or because of high lipid content (Benemann, 1996).

2.5.2.4. Bioflocculation

Bioflocculation is essentially a biological process, although influenced by the chemical environment in which the algae find themselves and which may be under some control by the pond operator (e.g. nutrient levels, pH, pO_2, etc.). By definition, no chemical additions are involved in the bioflocculation process, although small dosages of flocculants may well be used in practice to facilitate or accelerate the overall settling. The general observation is that microalgae cultivated in conventional (unmixed) oxidation ponds remain suspended for long periods after removal from the cultivation system, exhibiting negligible or very modest

sedimentation rates. Thus, tests for settling of microalgae cultures from such ponds were generally unsuccessful. The reason for this is rather easy to understand. The pond environment, particularly unmixed oxidation ponds, selects against any algae that settle, and thereby are removed from the photic zone. Even high rate ponds, where continuous mixing prevents ready settling of algae, does not provide any selective advantage to any algae that exhibits bioflocculation. Indeed, even in high rate ponds, the algae may settle out, particularly in larger ones with poor hydraulics and "dead zones". Thus, these ponds do not provide any reason for the algae to settle, and settling (bioflocculation) can actually be a detriment (Benemann, 1996).

Nevertheless, algal settling is a well known phenomenon. Algae settle in lakes and oceans, often through a process of bioflocculation, and this is a major factor in their ecology and life cycle. Even in oxidation ponds settling is a well known phenomenon. Indeed, oxidation ponds are normally designed with additional ponds, above and beyond those strictly necessary for photosynthetic oxygenation, based on the observation that algal concentrations actually decrease in these final "polishing" lagoons. Obviously, even without being observed, algae settle, possibly after having exhausted available nutrients. Indeed, settling appears correlated with nutritional deficiencies. Thus, N limited algal cultures often clump and settle, although this phenomenon has neither been widely studied nor reported. However, these phenomena were neither clear-cut nor reproducible. Due to nutrient regeneration, the polishing lagoons could exhibit large secondary blooms of algae, or little or no settling would take place for long periods. Nevertheless, there was considerable interest, and some fundamental and applied work in bioflocculation for algae harvesting and removal has been reported (Sobeck *et al*., 2001; Suh *et al*., 1997; Yahi *et al.*, 1994).

The first application of natural or spontaneous settling ("bioflocculation" in this terminology) as a method to actually remove microalgae from the effluents of conventional oxidation ponds was carried out at the Woodland, California, ponds (Koopman *et al*., 1979, 1980). Basically the method involved a long (typically 20 days) fill and draw cycle of a terminal settling pond. The process worked remarkably well: removal efficiencies for chlorophyll of 80% or higher were achieved in 10 out of 15 runs, and in 4 cases removal efficiencies were over 95%. However, the process could not meet the exacting requirements of wastewater treatment, due to lack of sufficient reliability and wind suspension of silt materials (which also count as suspended solids). Of course, such long cycle times would be impractical in any actual algae recovery process for biomass production.

The obvious advantage of bioflocculation for algal harvesting is that it has low capital and operating costs, if the algae exhibit sufficiently high sedimentation rates (>20 cm/h) (Benemann *et al*., 1978, 1982). However, up to now only modest successes have been achieved in controlling this phenomenon, which in most cases is not observed, and when it is observed cannot be reproduced, and when it is reproduced cannot be scaled-up, and when it is scaled-up is not sufficiently reliable to allow year-round, high (>95%) removal efficiencies, producing a 50 to 100 fold concentrate (1.5 - 3%).

2.5.2.5. Autoflocculation

Autoflocculation is phenomenologicaly similar to bioflocculation: after being removed from the pond, the algal culture flocculates and settles. However, mechanistically autoflocculation depends on the chemical flocculation of the algae by precipitation of calcium and phosphate ions in the pond medium. The term "autoflocculation" was first used by Golueke and Oswald (1965) who observed that during active photosynthesis in shallow ponds the entire algal

culture would settle to the bottom. Autoflocculation was caused by the chemical flocculation of the algae due to the precipitation of calcium, magnesium, carbonate, and phosphate as a result of the high pH in the ponds.

A study at the University of California, Berkeley, by Nurdogan *et al*. (1995), studied the effect of lime addition on the settling of *Micractinium* cultivated in the same ponds studied earlier by Benemann *et al*. (1980). They found that a concentration of 60 mg/l of CaO added to the pond increased phosphate removal from about 46% (without lime) to essentially 100%, due to the precipitation of various phosphate salts. Addition of 60 mg CaO/l also increased the size of the *Micractinium* flocs and their settleability from 70% to 95% in a 24 h settling test.

Flocculation of sewage grown algae by lime addition was studied extensively over two decade earlier (Folkman and Wachs, 1973). These authors have concluded that the main effect of lime addition was to generate positively charged magnesium hydroxide particles, which neutralized the algal surface charges, resulting in flocculation. Ca and Mg ions themselves were not effective. Sukenik and Shelef (1984) and Sukenik et al. (1985) studied autoflocculation in some detail in small outdoor ponds in a 2.25 mM $CaCl_2$ and 0.6 mM $MgSO_4$ enriched medium. Flocculation was induced by stopping CO_2 supply, which raised pH (from about 7 to 9), and mixing, which resulted in a rapid clearing (95%) of the culture, with loss of almost all the phosphate and some of the Ca and alkalinity. This was a light mediated pH effect, dependent on the presence of both Ca^{2+} and PO_4^{2-} ions. Laboratory experiments demonstrated that Ca (in the absence of Mg) was effective in autoflocculation at pH above 8.5, but only in the presence of PO_4. Mg mediated autoflocculation and required a higher pH, but no phosphate. They proposed that positively charged $CaPO_4$ particles neutralized the algal charges, causing flocculation.

In some cases, this phenomenon is associated with elevated pH due to photosynthetic CO_2 consumption, corresponding to precipitation of inorganic salts, mainly calcium phosphate, which causes the flocculation (Sukenik and Shelef, 1984).

2.5.2.6. Autoconcentration

Autoconcentration are processes by which motile algal cells self-concentrate due to their motility and tendency to swim toward the light (phototaxis) or some other chemical attractants (or away from repellants). The coordinated beating of the flagella in these algae allows the achievement of considerable swimming speeds, over one cm/min. In principle, such a swimming speed would be sufficient to achieve rapid autoconcentration. Laboratory-scale experiments were promising, with 99.9% of the motile cells being removed from the bulk solution, but operational problems, such as the settling of non-motile cells, hydraulic problems, low concentration factors, etc., do not allow a clear interpretation of the results Benemanm, 1996).

Nakajima and Takahashi (1991) studied phototaxis by *Euglena* as a method of algal separation from wastewaters. The effluent from an *Euglena* culture was passed through a "photoclarifier", a darkened vessel with a small illuminated area where the cells congregated. The medium near the light was recycled to the culture vessel and that at the far end (in the dark) was discharged. In one configuration, essentially no cells were observed in the effluent after stabilization of the system (appx. 20 days). They speculated that this was due to selection for highly phototactic cells. Of course, such complete recycling resulted in a very high density in the growth vessel and thus the increase in recycle efficiency could be equally well due to increasing light limitation.

Mohn (1988), in his review of algae harvesting, briefly mentioned phototactic separations, dismissing it by stating that the process would probably involve hundreds of square meters of controlled illumination and consequently to be economical, applications would require a most exotic and valuable product. However, such a negative attitude is not warranted by the (few) facts and autoconcentration is worth future investigation. In the context of CO_2 mitigation and fuel production, motile algae may be of some interest, in particular *Botryococcus braunii.*

2.5.3. Filtration of Unicellular Algae

There are three major problems with the filtration of unicellular microalgae: the very small size of the cells, their near (for practical purposes) spherical shape, and the gelatinous and other extracellular material normally present on the algal cells, which result in deformation on the filter surface. These properties result in generally poor filtration, due to the plugging of the filters and/or breakthrough of algal cells. Extensive studies have been carried out on algal filtration, most without much success. Filtration is best if carried out with filamentous or large colonial algae, which can be readily handled by large mesh screens (Molina *et al*., 2003).

There are many variations on the basic filtration process, involving a large variety of filter materials, including regenerable pre-coats; use of pressure, vacuum, centrifugal forces (basket centrifuges); and methods to keep the filter surface from clogging (blinding) by hydrodynamic means (shear forces). Shelef *et al*. (1984) describe some of the basic filtration systems. Mohn (1980, 1988) carried out research on various filtration systems, particularly for recovery of the alga of *Coelastrum*, a relatively large algal colony of open structure, and would be expected to exhibit good filtration. Several filtration techniques proved effective for this alga, but not for smaller *Scenedesmus* or *Chlorella* colonies.

2.5.4. Air Flotation Systems

The principle of air flotation is to introduce tiny bubbles of air, which transport solid particles in suspension to the surface of a pond or tank, where they can be removed by skimming or some other means. Dissolved air flotation (DAF) has been used widely in water processing for more than 50 years. More recently, amongst other applications, induced air flotation (IAF) systems have been developed to remove algae from suspension.

2.5.4.1. Dissolved Air Flotation

In DAF, tiny micro-bubbles (<100 μm) lift suspended particles to the surface of the chamber. These bubbles are created by injecting high pressure recycled water, saturated with dissolved air (using blowers or compressors), into the DAF chamber. When the high-pressure water encounters the atmospheric pressure of the water in the chamber, the excess air is released as micro-bubbles that attach to suspended particles such as algae, causing them to float to the surface. Algal float containing 4–6% solids can be obtained by skimming off the section of the float not submerged in the medium. Prior to entering the DAF chamber, the influent usually passes through a flocculation chamber where flocculants are added and generally some sort of mixing device is used to create floc sizes of the order of 30-100 μm, similar to that of the bubble sizes (Edzwald *et al*., 1990, 1993, 1995).

2.5.4.2. Induced Air Flotation

The Jameson Cell operates on the principle of IAF and recent studies and pilot operations outline significant advantages over conventional flotation technologies for algal removal operations. As in DAF processes, flocculants are first added to the influent and the mixture is introduced to the top of the cell. A portion of clean effluent is recycled back to the cell as a liquid jet which entrains air in a similar fashion to a hose plunging into a bucket of water. In

this process, the energy of the fluid induces air rather than requiring an external compressor or blower. The cell chamber acts as a disengagement zone allowing the aerated particle aggregates to float to the surface to form a sludge layer. The sludge layer overflows over a weir structure into a collector, while the "particle-free" effluent passes through to subsequent treatment or is discharged (Jameson, 1999).

2.5.5. Electrolytic flocculation

Electroflotation uses electrolysis only to produce gas bubbles which uplift the flocculated algae to the surface. The electrodes are placed horizontally, covering the bottom of the flotation tank. Flocculation of the algae has to be performed with flocculants such as alum which makes the electroflotation technique similar to dissolved air flotation (DAF). With electrolytic flocculation, on the contrary, no flocculants are needed and the electrodes are placed vertically.

The electrolytic flocculation experiments are based on the principle of the movement of electrically charged particles in an electric field. Micro-algae have a negative surface charge which causes them to be attracted by the anode during the electrolysis of the algal suspension. Once they reach the anode they lose their charge which make them able to form algal aggregates. As the electrolysis of water means the production of hydrogen and oxygen gas at the electrodes, the bubbles produced at the anode (oxygen) rise to the surface taking with them algal aggregates or flocs, which can be skimmed off easily. The electrolysis leads in this way to the flocculation and flotation of the algae at the same time, without the usual addition of chemical flocculants.

This new technique consume relatively little energy (0.3 $kWh.m^{-3}$) and is easy to control and applicable to various groups of micro-algae but, most important, it results in an efficient separation of the algae (> 90%). Moreover, since it is not contaminated with toxic flocculants,

the harvested algal biomass can afterwards be used for different purposes such as algal feed and food. Further research on electrolytic flocculation would have to look for application development at the industrial scale since only small-scale experiments have been carried out up to now (Poelman *et al*., 1996).

Chapter III

3. MATERIAL AND METHODS

3.1. MATERIALS

3.1.1. Algae species and culture medium

Several algae species were considered for this project and the decision about the algae strain was taken by the responsible ASU Algae Lab researchers. A *Chlorella* sp. strain was chosen due to high oil content, fast growing and resistance to contamination characteristics. *Chlorella* cultures were obtained from Arizona State University Algae Research Laboratory and the specific isolated strain was not identified for commercial and patent protection reason.

Chlorella is a green coccoid species of single cell, belonging to *Chlorophyta* (phylum), *Chlorophyceae* (class), *Chlorococcales* (order) and *Oocystaceae* (family). Cells are slightly ellipsoidal to spherical and mother cells are approximately spherical. The cell wall is thin. During spore formation, the cell wall does not undergo gelatinization, but breaks up into 2 fragments. The chloroplast is cup-shaped or rarely girdle-shaped. The pyrenoid is distinct, covered with saucer-shaped starch grains. Vacuoles can be seen when the cells are young. Lipid granules are present when the cells are old. Reproduction occurs by 2 or 4 (or rarely 8 or 16) autospores. Young autospores are always slightly ellipsoidal. Fragment of the mother-cell wall is broadly elongated or irregularly triangular, yet always concave, two pieces contact with one of the ends. They persist separately from the released autospores and lie freely in the culture medium. Dimension of the cells is typically of 3-6 µm, sporangia up to 10 µm (Richmond, 2004).

Table 3.1 shows the composition of the nutrient media used for growing the selected *Chorella.* BG 11 is a growth medium that has been used extensively for freshwater green algae and cyanobacteria.

Table 3.1: BG 11 composition (adapted from Richomond, 2004)

Chemical Reagent	BG11
$NaNO_3$ ($g.l^{-1}$)	1,5
$K_2HPO_4 \bullet 3H_2O$ ($g.l^{-1}$)	0,04
$MgSO_4 \bullet 7H_2O$ ($g.l^{-1}$)	0,075
$CaCl_2 \bullet 2H_2O$ ($g.l^{-1}$)	0,036
Citric acid ($g.l^{-1}$)	0,006
Fe-Ammonium citrate ($g.l^{-1}$)	0,006
EDTA, 2Na-Mg salt ($g.l^{-1}$)	0,001
Na_2CO_3 ($g.l^{-1}$)	0,02
H_3BO_4 ($\mu g.l^{-1}$)	2,86
$MnCl_2 \bullet 4H_2O$ ($\mu g.l^{-1}$)	1,81
$ZnSO_4 \bullet 7H_2O$ ($\mu g.l^{-1}$)	0,222
$Na_2MoO_4 \bullet 2H_2O$ ($\mu g.l^{-1}$)	0,391
$CuSO_4 \bullet 5H_2O$ ($\mu g.l^{-1}$)	0,079
$Co(NO_3)_2 \bullet 6H_2O$ ($\mu g.l^{-1}$)	0,494
Adjusted final pH	7,4

3.1.2. Photobioreactors

It has been seen that microalgae can grow in controlled conditions in the laboratory. Temperature, pH, and other important variables can be well controlled compared to the large-scale systems done in open ponds. Growth of *Chlorella* was carried out in two different types of photobioreactors as described below.

3.1.2.1. Small scale bubble column reactor

The batch growth experiments were performed in glass tubes contained in a thermal reactor consisting of a rectangular plexi-glass tank. The volume of the thermal reactor was of 94liter and the walls had a thickness of 1.27-cm. Glass columns of diameter 50 mm (750 ml volume) were used as fixed volume photobioreactors to hold the microalgal cultures.

An Immersion Circulator (VWR Scientific Products Model 71) with Analogic Controller was used to control the water bath temperature to the desired level in the reactor for the temperature experiments. In order to control the pressure coming out of the cylinders, CONCOA High Purity Gas Valves (Virginia Beach, Virginia USA) were used. CO_2 and compressed air were blended for the batch experiments using a Cole-Parmer Gas Proportioner flow meter (Vernon Hills, Illinois, USA) with a steel float. The CO_2 / air mixture was bubbled at a rate of 9.6 L/min with a constant supply of 5% CO_2. The blended gas was bubbled into the columns using glass diffuser tubes (Capillary) through Vinyl tubing. A pH meter and a thermometer were used to measure pH and the temperature in the columns. Six cool 45 W fluorescent lamps fixed to the panel placed adjacent to the plexi-glass tank provided light 24 hours a day for the experiments. A schematic diagram of the bubble column photobioreactor system is shown in Figure 3.1.

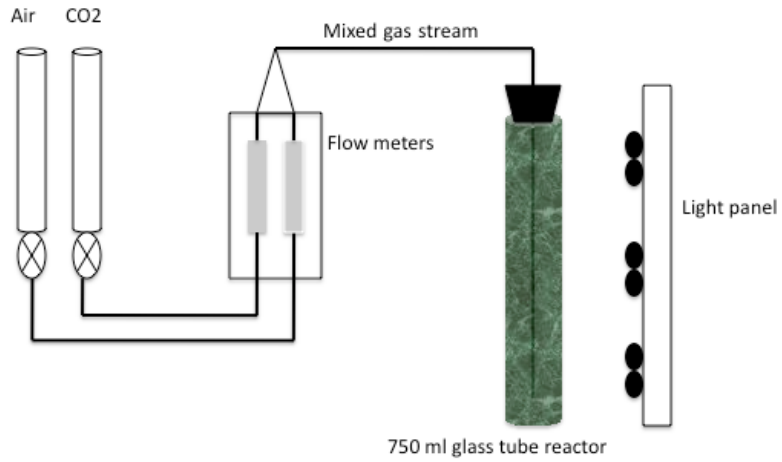

Figure 3.1: Small scale bubble column reactor diagram

3.1.2.2. Flat-Plate Photobioreactor

A schematic diagram of the flat-plate photobioreactor system is shown in Figure 3.2. The lab-scale flat-plate photobioreactor was made from 1.27 cm thick plexi-glass sheets with a culture volume holding capacity of 10 L.

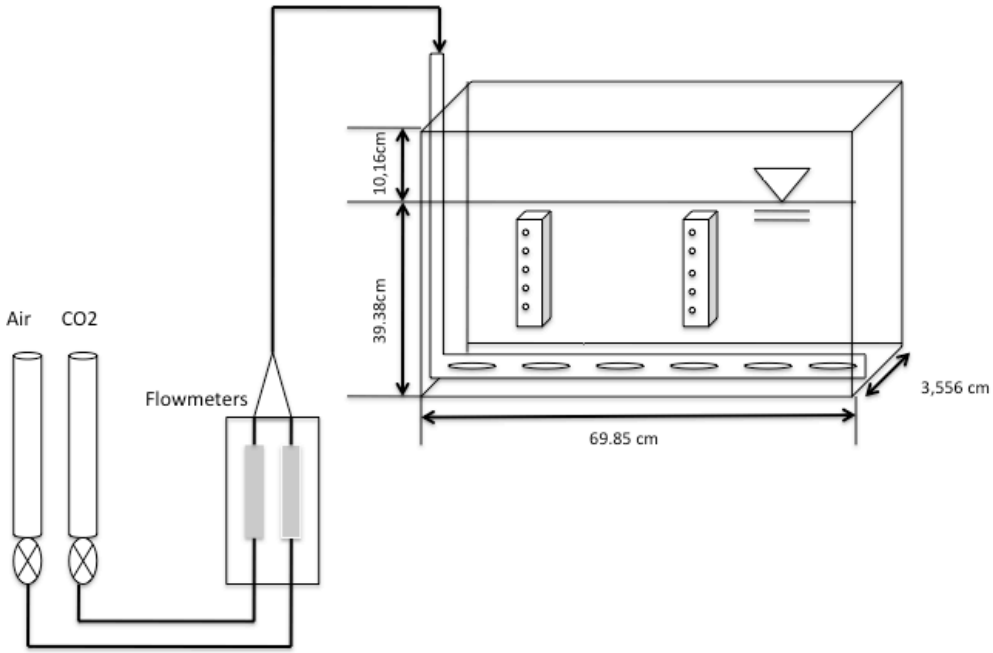

Figure 3.2.- Flat-Plate Photobioreactor diagram

The tank consisted of a rectangular chamber 69.85 cm long, 3.556 cm wide and 40.132 cm in height. A perforated 0.48 cm (inner diameter) fluoropolymer tubing with 0.05 mm equally spaced holes with a length of 68.58 cm was used to bubble the gas mixture in the reactor, which was attached to 40 mm steel tubing with a length of 45.72 cm.

Steel weights were used to hold the tubing. A gasket groove was placed in order to provide maximum air tightness with a dimension of 0.635 cm wide and 0.158 cm deep. The flat-plate

photobioreactor was fixed to two Uni-Strut vertical supporting structures, 1.22 m tall. A Cole-Parmer gas proportioner flow meter was used to mix the house air and compressed CO_2 source using a CONCOA regulator (Virginia Beach, Virginia, USA). The CO_2 / air mixture was bubbled at a rate of 9.6 L/min with a constant supply of 5% CO_2. The gas flow rate was maintained at this level in order to keep the culture suspended in the medium. Six cool 45 W fluorescent lamps fixed to the panel placed adjacent to the flat plate photobioreactor provided light 24 hours a day for the experiments.

3.2. ANALYTICAL METHODS

3.2.1. Algae culture growth and screening

3.2.1.1. Cultivation of *Chlorella* sp.

The same *Chlorella* sp. strains were used in this project to seed the batch growths in the bubble column and in flat plate photobioreactors. The stock culture was incubated in 100-150 ml Erlenmeyer flasks containing 50 ml of fresh BG-11 medium and then covered with sponge flask covers in order to avoid contamination. The Erlenmeyer flasks were then placed on an orbital shaker, under a light intensity of approximately 140 µmol/(m^2.s), at a room temperature of 26°C for a week or 15 days until the culture turns deep dark green color. The cultures were then centrifuged at a temperature of 25°C and at a rotational speed of 5000 rpm for 6 minutes. After the centrifugation, the supernatants were discarded, and the cultures were rinsed and re-suspended in a fresh BG-11 medium. The fresh cultures were then placed in the glass column and in the flat plate photobioreactors each with BG-11 medium.

3.2.1.2. Light intensity, pH and temperature

In order to evaluate the amount of light intensity striking the microalgal cells, the light intensity was measured using a Quantum Meter, Model LI-189 LI-COR (Lincoln, Nebraska, USA), which had a quantum sensor (Silicon Photodiode). As quantum sensors measure photosynthetically active radiation (PAR) in the 400–700 nm waveband, the light meter was used for light intensity measurements in the batch reactor and the flat-plate photobioreactor.

In order to monitor the temperature of the culture, temperature measurements were made using a Cole-Parmer mercury thermometer. Temperature measurements for the batch photobioreactor and the flat-plate photobioreactor were taken directly by inserting the thermometer inside the culture. The temperature of the plexi-glass water bath was controlled using the Immersion Circulator (VWR Scientific Products Model 71) with manual controller.

pH was measured for all the batch and flat-plate photobioreactor experiments using a Corning pH meter 340. Monitoring of pH was necessary in order to keep the cultures in good conditions.

3.2.1.3. Evaluation of cell density and dry mass

A useful method for cell density quantification is the gravimetric method by cell dry weight determination. It was observed that cell dry weight evaluation is less sensitive to the physiologic state of the cell than direct counting, but a calibration curve is essential for fast routine assays. A calibration curve of cell dry weight *versus* optical density is very useful, because absorbance is very easy to read and usually the different culture conditions do not affect the linear proportionality (Rocha *et al.*, 2003).

The dry weight was measured by filtering an aliquot (10 mL) of culture suspension on pre-weighted Whatman GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.). A suction pump was used to filter the samples and after filtration the filters were dried in a Cole Laboratory oven (Parmer Instrument Company, USA), at 105-110 °C, for approximately 24 hours. The filter was taken and cooled to room temperature in a desiccator and was weighed in an analytical and precision balance (Sartorius BP 110 S, Germany) to get the final weight (Hu *et al*. 1998). The difference in the weights of the filter, after filtration and before filtration, divided by the sample volume filtered gives the dry mass of the microalgae culture. The dry mass values of all algal species were determined on triplicate culture samples.

Absorbance was determined by a UV/VIS Spectrophotometer (JenaWay 6300 Spectrophotometer, U.K.) at a wavelength of 680 nm. Each sample was diluted to give an absorbance in the range of 0.1–1.0 if the optical density was greater than 1.0. This procedure was adopted because the biomass could be underestimated if the optical density would be out of the linear range. A plot of the optical density at 680 nm and dry mass in g/L was developed. The calibration curve that has resulted from this proceeding were regularly checked.

3.2.2. Algae characterization

3.2.2.1. Surface functional groups

Cells were harvested by filtering an aliquot (10 mL) of culture suspension on Whatman GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.). A suction pump was used to filter the samples and after filtration a sample of the algae paste was deposited on a silicon window (Crystran

Ltd., Poole, UK). The algal suspension was allowed to partially dehydrate for 2 h at room temperature on the window (Hirschmugl *et al.*, 2006). The level of dehydration was the minimum required to reduce the dominance of the H_2O absorption. *Chlorella* surface spectra were determined with a Fourier transformation infrared spectroscope (Thermo Nicolet Avatar 360 FT-IR, USA).

3.2.2.2. Particle size measurement

The algae medium culture was filtrated with Whatman GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.) in a vacuum pump and then soaked in a beaker with a electrolyte background solution of nitrate potassium KNO_3 (0,1 M). Square cuvettes of 10 mm (Brookhaven Instruments Corporation BI-SCP) with a sample holding capacity of 4.5 ml were used to hold the samples. The size of the algal cells was measured using a light scattering unit (Brookhaven Instruments, ZetaPALS Particle sizing Software). Aas (1996) determined 1,047 as the refractive index value for *Chorella* through flow cytometry techniques and this value was inserted in the ZetaPALS software.

3.2.3. Zeta potential measurement

3.2.3.1. Overview

Zeta potential is measured by applying an electric field across the dispersion medium containing the particles. When an electric field is applied across an electrolyte, charged particles suspended in the electrolyte are attracted towards the electrode of opposite charge.

Viscous forces acting on the particles tend to oppose this movement. When equilibrium is reached between these two opposing forces, the particles move with constant velocity. The velocity is dependent on the strength of electric field or voltage gradient, the dielectric constant of the medium, the viscosity of the medium and the zeta potential. The velocity of a particle in a unit electric field is referred to as its electrophoretic mobility. Zeta potential is related to the electrophoretic mobility by the Henry equation, as follows:

$$UE = \frac{2.\varepsilon.z.f(\kappa a)}{3.\eta} \quad (1)$$

|

where *UE* = electrophoretic mobility, *z* = zeta potential (mV, ε = dielectric constant, η = viscosity ($Pa.s^{-1}$) and *f(κa)* = Henry's function. The units of *κ*, termed the Debye length, are reciprocal length and (*κ-1*) is often taken as a measure of the "thickness" of the electrical double layer. The parameter *a* refers to the radius of the particle and therefore *κa* measures the ratio of the particle radius to electrical double layer thickness. These electrophoresis determinations of zeta potential are made in aqueous media and moderate electrolyte concentration. *f(κa)* in this case is 1.5, and this is referred to as the Smoluchowski approximation (Henderson *et al.,* 2008).

3.2.3.2. Apparatus and procedure

Algae solutions produced in both photobioreactors used for z potential calculations were first filtrated with Whatman GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.) in vacuum pump and then soaked in a beaker with an electrolyte background solution of KNO_3 (0,1M). Then a beaker containing 60 mL of these algal suspension and a magnetic stirrer bar were placed on a

magnetic stirrer. The pH values of the algal suspensions were adjusted to from 2 to 12 with varying amounts of 0.1 M HNO_3 and 0.1 M KOH that were added to the beaker. The pH values of the algae samples were determined using the pH meter pHI 250 Handheld (BECKMAN-COULTER, USA).

The zeta potential was measured with a Zeta Potencial analyzer ZetaPALS (Brookhaven Instruments Corporation, UK). To measure the particle electrophoretic mobility, this instrument uses a phase analysis light scattering (PALS) based on the shifted frequency spectrum. Square cuvettes of 10 mm (Brookhaven Instruments Corporation BI-SCP) with a sample holding capacity of 4,5 ml were used to hold the samples. The algae samples were then illuminated by a cross focused laser beam. It was assumed that the algae cells were not damaged by the beam as it was only applied to them for a short time (Poochinda and White, 2003).

The ZetaPALS gives estimates and standard deviations of the zeta potential directly, and all measurements were conducted in triplicate. The general accuracy of the ZetaPALS was determined using standard solutions. The point of zero charge or iso-electric point (IEP) had been established.

3.2.4. Jar Testing

3.2.4.1. Overview

The jar test is a common laboratory procedure used to determine the optimum operating conditions for water or wastewater treatment plants. Jar testing traditionally has been done

on a routine basis because it allows adjustments in pH, variations in coagulant or polymer dose, alternating mixing speeds, or testing of different coagulant or polymer types, on a small scale in order to predict the functioning of a large scale treatment operation. A jar test simulates the coagulation and flocculation processes that enhance the removal of suspended colloids and organic matter, which can lead to turbidity, odor and taste problems. This usually consists of a set of six beakers that can be stirred simultaneously at known speeds. Equal volumes of water to be treated are placed in the beakers. Different doses of flocculant are added to each beaker, followed immediately by a brief period of rapid stirring. A period of slow stirring to allow orthokinetic flocculation to occur is then performed. The stirrers are then stopped and any flocs formed are allowed to settle (Gregory, 1983).

3.2.4.2. Samples, apparatus and procedure

The *Chorella* suspension (B1) was cultivated in the flat-plate photobioreactor according to the conditions described previously and it was left to grown for two weeks. A total volume of 8 L was produced.

A standard Phipps and Bird six-place gang stirrer was used for the investigation (Phipps & Bird PB-900™, VA, USA) and for setting time intervals a manually-operated timer was used. The light base provides a full and clear view of all the beakers simultaneously and the fact that fluorescent bulbs were used did not change the temperature of the sample significantly. A black background was placed behind the jar test to give a better view of the flocculation process.

Six square two liter beakers (HACH Company CO, USA) were used to limit the formation of vortex flow in which the particles rotate in the same position relative to each other.

Major preceding steps for jar-testing were adopted from APHA, AWWA, and WEF (1992) and the proposed analytical plan is summarized in Table 3.2.

Table 3.2: Jar testing experimental matrix

Experiment	Jar #	Variables	
		Desired pH	Concentration of background electrolyte
Experiment 1	1	2	1 mM KNO_3
	2	4	
	3	6	
	4	8	
	5	10	
	Control	-	
Experiment 2	1	2	10 mM KNO_3
	2	4	
	3	6	
	4	8	
	5	10	
	Control	-	
Experiment 3	1	2	100 mM KNO_3
	2	4	
	3	6	
	4	8	
	5	10	
	Control	-	

- A batch was siphoned off to a 25 L bucket and diluted with deionized water. 20 liters of culture were prepared for each. The main driver for this dilution was getting more volume of solution to run three experiments for batch.
- Using a 1000 mL graduated cylinder, 1000 mL of solution was measured and put into each of six 2000 ml Lab Jars. 50 ml of the electrolyte background solution previously prepared (1 mM, 10 mM and 100 mM KNO_3) was also added.
- After the solutions have been prepared, the beakers were placed under the stirring paddles. The paddles were lowered into the beakers.
- KOH 0.1 M and HCl 0.1 M were then added until reaching desired pH. 40 rpm for 2 minutes was used for agitation/stirring the solutions.
- According to the ASTM 2035-08 Standard Practice, the suspensions were rapidly mixed at 120 rpm for 1 minute, then at 20 rpm for 20 minutes, and left to settle for 30 minutes.
- After the settling period, paddles were removed and a probe Hach sensION156 (Hach company CO, USA) was introduced for measuring conductivity in all six jars.
- By means of a pipette, an aliquot of the supernatant was withdrawn from each jar at a half point depth of the sample to a Fisher-Scientific® Fisher*brand*® Standard 10-mm pathlength quartz cuvettes with a sample holding capacity of 3 ml.
- The cuvettes were analysed for absorbance at a wavelength of 680 nm (Jenway 6300 UV/Vis spectrophotometer, Jenway, UK) and algae removal was calculated according to equation (2):

$$P = \frac{C_0 - C}{C_0} \times 100 \quad (2)$$

Where P is the percentage of algae removed from the culture; *C* is the algae concentration after the flocculation test ($g.L^{-1}$) and C_0 is the algae concentration after the flocculation test in the control jar ($g.L^{-1}$) (Sukenik and Shelef,1994).

Chapter IV

4. RESULTS AND DISCUSSION

4.1. ALGAE CULTURE GROWTH AND SCREENING

Growth experiments were performed with *Chlorella* strains from the ASU Algae collection in the two types of photobioreactors described above. The bubble column photobioreactor was used mainly to grow small samples used for zeta potential determinations and to seed flat plate photobioreactors. It was easily operated with a constant supply of 5% CO_2 and temperature was maintained at 24°C. Initial and final pH values of the medium were 5.65 and 7.05, respectively, and the light measurements indicated an average value of 160 µmol/(m^2.s). Flat plate photobioreactor trials were also tracked operating with a constant supply of 5% CO_2 and temperature maintained at 24°C. Initial and final pH values of the medium for Flat Panel Batch 1 were of 6.1 and 7.9, respectively. The average light radiation was of 160 µmol/(m^2.s).

Cell density and biomass were measured more easily by optical density than by direct counting of cells or by cell dry weight. Therefore, relationships between optical density and cell density and optical density and cell dry weight were established by linear regression. For the bubble column photobioreactor, optical density was strongly related with biomass concentration (cell dry weight) (R^2 = 0.987; $p < 0.001$) (Figure 4.1). Therefore, the values of optical density were used to calculate the related biomass of *Chlorella* samples in jar tests experiments, according to the equations established in this study.

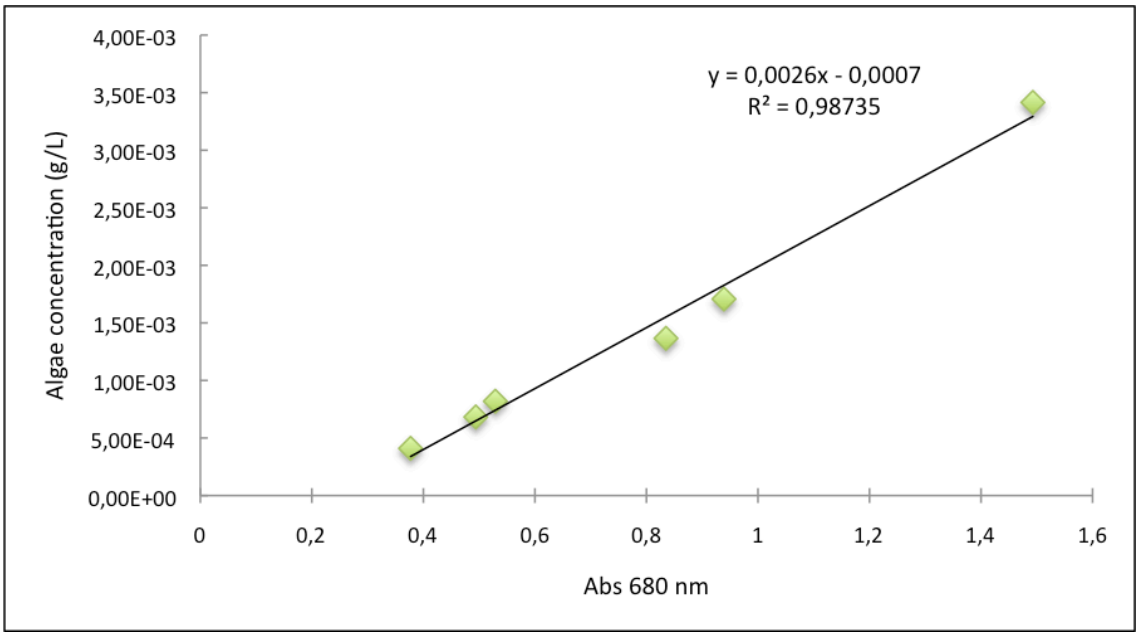

Figure 4.1: Calibration curve of optical density at 680 nm and cell dry weight

Even thought *Chlorella* is a fast growing algae and cultures seemed resistant to contamination, the set of experiments concerning the evaluation of different life stages were not totally successful since the brown stage (high oil phase) was not achieved. The applied "stressing the algae to increase oil content methodology", property of the ASU Algae Lab, did not worked out in the two tested trials with this *Chlorella* strain and the time frame for this investigation was short for preparing more trials. Increasing the oil content revealed some drawbacks, which may be due to contaminations and light stress.

4.2. ALGAE CHARACTERIZATION

4.2.1. Particle size measurement

Figure 4.2 shows an example of particle size distribution obtained for a *Chlorella* sample produced in the bubble column photobioreactor with a concentration of 6.22 g/L of cell dry weight. The particle size distribution for the flat plate photobioreactors batches was similar, indicating that the concentrations of biomass achieved in this photobioreactor were not relevant for cell size. The size distribution showed a range of particles from 2.6 to 3.6 nm with a single size mode of approximately 3.1-3.2 µm. As shown in Figure 4.2, the particle size can be described with a log-normal size distribution.

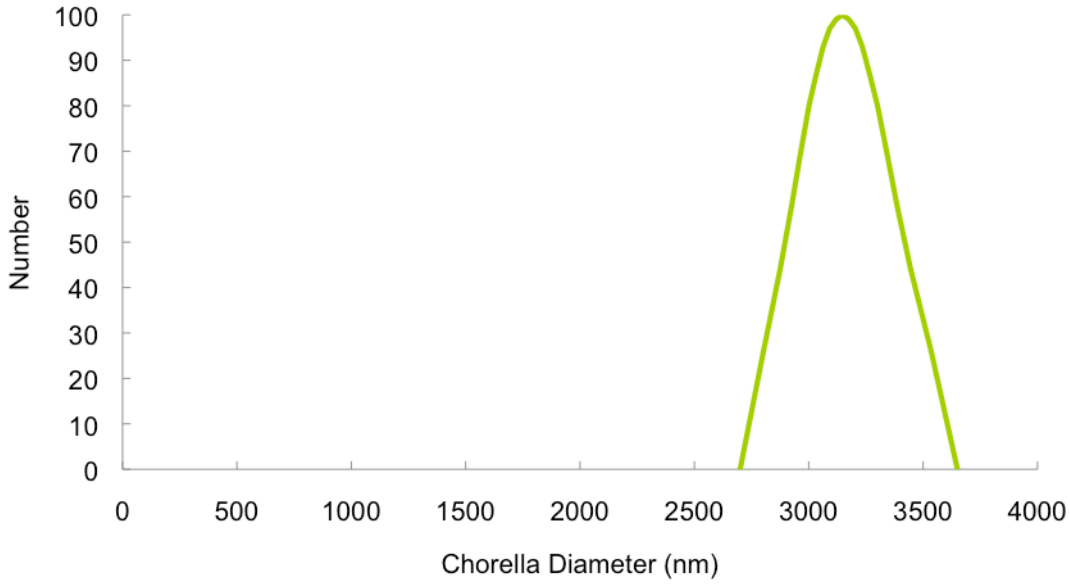

Figure 4.2: Log-normal size distribution for the ASU *Chlorella* strain

4.2.2. Surface functional groups

Identification of the functional groups on algae surface was performed by FTIR and Figure 4.3 demonstrates the FTIR spectrum for the algae cultivated in the flat plate photobioreactor.

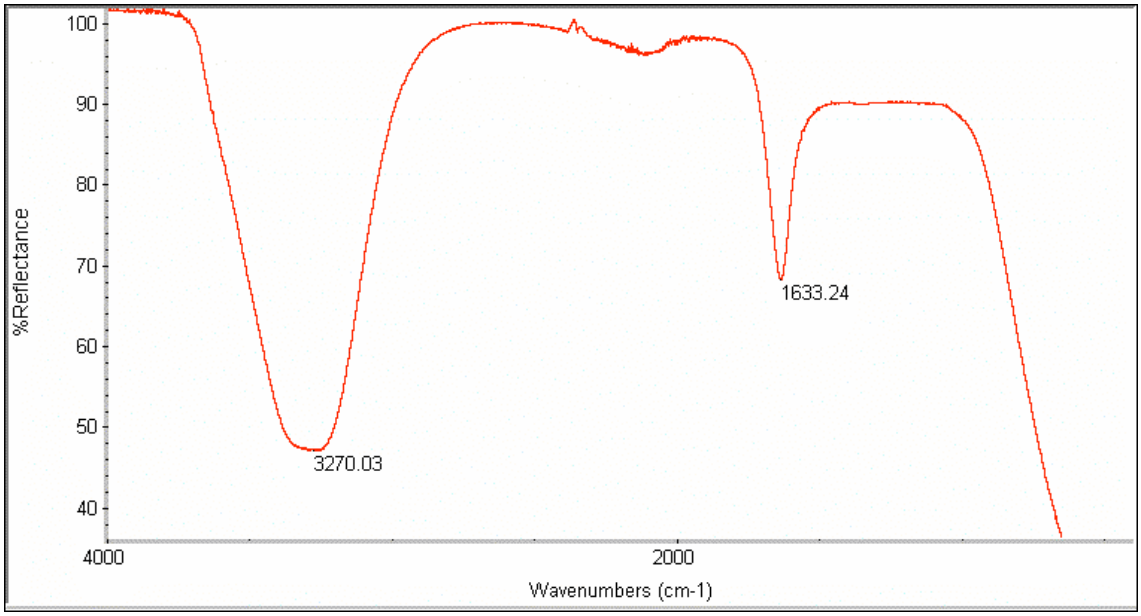

Figure 4.3: FTIR spectrum of *Chlorella* sp. cells cultivated in the flat plate photobioreactor

It was confirmed that N–H stretching occurred at 3270.03 cm^{-1} and the presence of amide (C-O) peak at 1633 cm^{-1}. The conjunctions between these peaks revealed that both protein and polysaccharide-like substances were the major constituents on algal surface. This FTIR spectrum indicated similar peaks to those obtained by Huang *et al*. (1994) and Liu *et al*. (1999).

4.2.3. Zeta potential measurements

The results shown in Figures 4.4 and 4.5 indicate that zeta potential of both the bubble column and flat plate photobioreactors changed significantly with the change of the broth medium pH values.

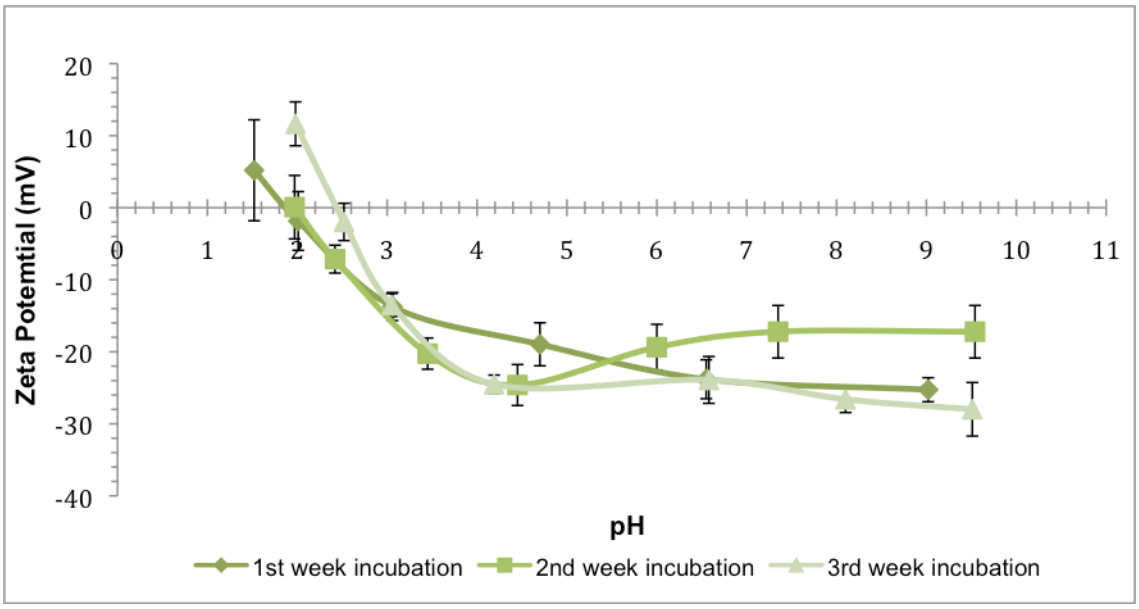

Figure 4.4: Zeta potential of *Chlorella* sp. cultivated in the Bubble column photobioreactor, in different life stages

Zeta potential values for this *Chorella* strain demonstrate that the surface charge is typically electronegative for pH ranges from 2.5 to 10 with values for all samples ranging between -2 and -35 mV. As the pH values were increased, the zeta potential decreases due to the increase in the OH^- activity and corresponding adsorption of the OH^- groups onto the algal cells (Phoochinda and White, 2003). Hence, the resulting suspensions are proved to be stable at ambient pH values, and the negative surface charge originates from the ionisation of functional groups on the algae cell wall surface, of which protein molecules are a major component of the membrane (Huang *et al*., 1999). The zeta potential reached a value of zero at a pH value of about 2 in all tests and that observation is consistent with other tests in which

the iso-electric point of charge was defined as raging between 1.5 and 3.5 (Liu *et al.*, 1999; Chen *et al.*, 1998; Huang *et al.*, 1999; Phoochinda and White, 2003).

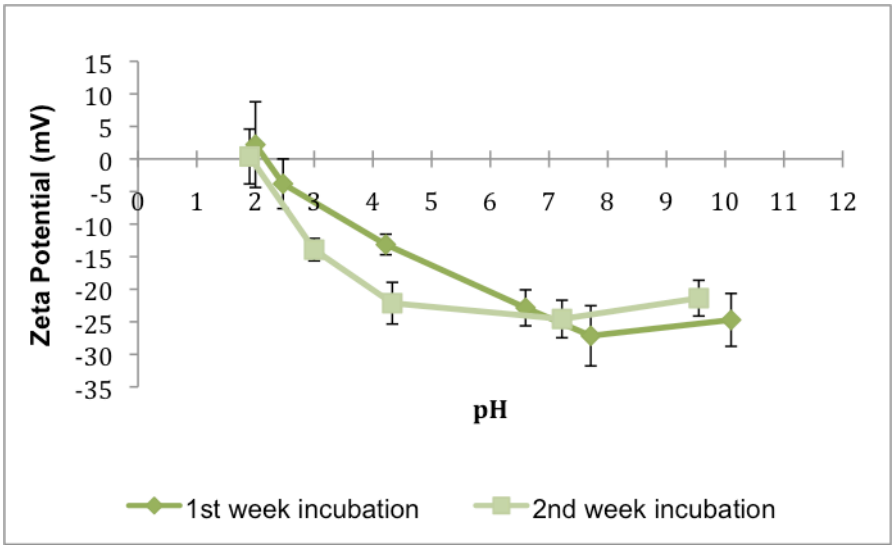

Figure 4.5: Zeta potential of *Chlorella* sp. cultivated in the Flat plate photobioreactor, in different life stages

Furthermore, zeta potential value has been demonstrated to change, depending on the algal growth phase. For instance, for the *Chlorella* sp. produced in the bubble column, zeta potential (Figure 4.4) was -23,8 mV during the initial growth phase, -26,5 mV in the log growth phase and -17,2 mV in the stationary phase. As it can be seen in Figure 4.5 the zeta potential for the flat plate production was only calculated for the initial growth (-24,5 mV) and log growth phase (-27,1 mV) and the differences were very small. Zeta potential for stationary growth phase was not measured due to the previous referred problems concerning contaminations. It has been suggested (Edzwald and Wingler, 1990; Konno, 1993) that the relation between the zeta potential and the algae growth phase is due to an increase or decrease of extracellular organic matter (EOM), dependent on when during the growth phase the algae are sampled, which in

turn generates a negative charge. During the log growth phase, the algae could be extremely active and as a result producing a larger volume of algogenic substances, which then act to increase the negative surface charge.

4.3. ALGAE REMOVAL - JAR TESTING

Jar tests were run to determine impact of pH in the separation of algae from water through coagulation–flocculation. Tests were conducted using different ionic strengths. Figure 4.6 confirms increasing conductivity with higher KNO_3 concentrations. The conductivity difference between the microalgae solutions with 1 mM and 10 mM KNO_3 was almost residual, ranging from 2500 μS/cm, for pH 2, to 1000 μS/cm, for pH from 4 to 10. The trial where 100 mM KNO_3 were added reached 4000 μS/cm near to the iso-electric point, and decreased to approximately 3000 μS/cm from pH 4 to 10.

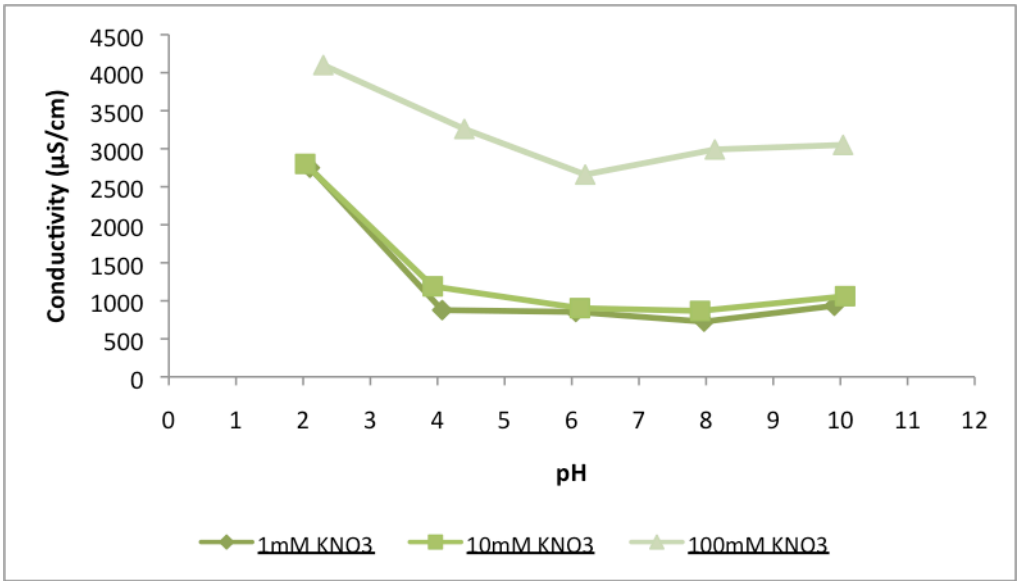

Figure 4.6: *Chlorella* sp. solution conductivities with different background electrolyte concentrations

As shown in Figure 4.7**,** a very low removal peak was observed for the three trials at approximately pH 2.0. Between 15% and 28.6% of algae removal were achieved. *Chlorella* sp.

cultures are known to have zero net surface electric charge for that range of pH levels (Figure 4.4 and 4.5) and consequently the probability of contact interactions between cells increases since no or low mutual electric repulsion forces are present.

Almost no removal was obtained for pH between 4 and 10. Sukenik and Shelef (1994) and Yahi *et al*. (1994) found flocculation zones with high removal rates for pH levels above pH 8.5. Autoflocculation has been reported as chemical flocculation of the algae due to the precipitation of calcium, magnesium, carbonate, and phosphate as a result of the high pH. Thus, this could mean that the concentrations of calcium, magnesium, carbonate, and phosphate were low in the cultivation medium at the time the jar test was performed.

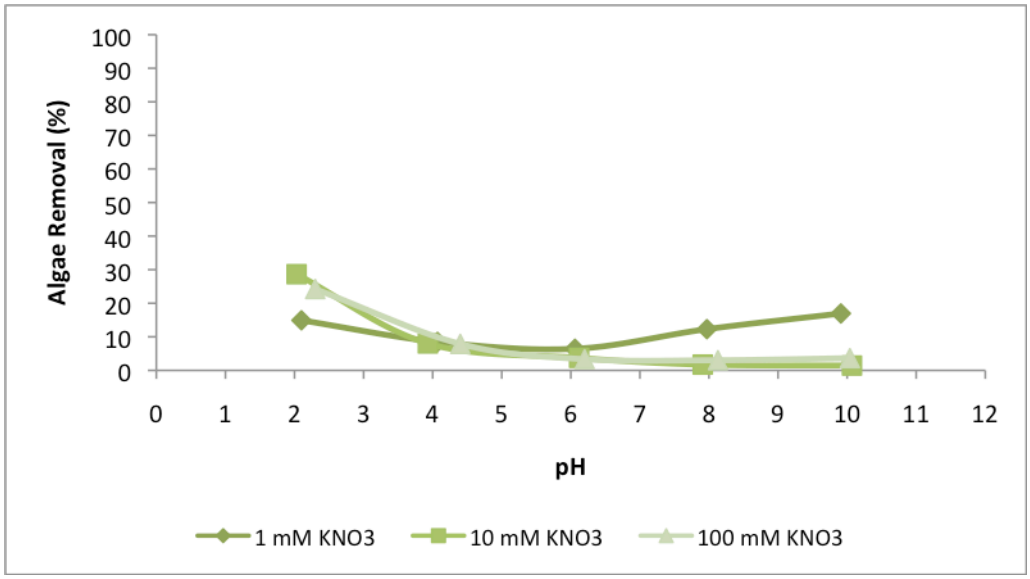

Figure 4.7: Percentages of *Chlorella* sp. removal as a function of pH values of broth medium

Furthermore, the increase in ionic strength should enhance the electrical-double-layer compression, thereby resulting in a decrease in repulsive force and zeta potential (Huang *el al*., 1999). However, it was found that algae removal was not significantly affected by ionic

strength. This phenomenon can be explained by the fact that algae cells are known to be able to react to perturbations caused by changes in the environment. The physiological ability of algae may allow the cells to counterbalance electrical neutralization as a result of the addition of a coagulant or other ions for flocculation (Pieterse and Cloot, 1997).

Chapter V

5. CONCLUSIONS AND FUTURE WORK

5.1. CONCLUSIONS

This project has extended previous *Chlorella* production characterization and harvesting studies. The following overall conclusions have been drawn:

- This work has highlighted the relationship between zeta potential and the coagulation and flocculation process. Comparison of all available data reveals a relationship between cell surface and zeta potential at different incubation stages. It is thus confirmed that *Chlorella* zeta potential is negative for a wide pH range and that would provide a stability of the solution. However, the incubation for the last stage (high oil content) was not successful demand and it is relatively poorly understood the influence of this last stage on zeta potential.

- It was also shown that coagulation/flocculation for this *Chlorella* strain with pH adjustment was not a robust harvesting method. Up to 28.6% removal was achieved for pH values close to the iso-electrical point. However, successful removal with this methodology relied heavily on high pH and presence of calcium, magnesium, carbonate, and phosphate. It is thus suggested that the concentrations of these cations were low in the cultivation medium at the time the jar tests were performed.

- It was found that algae removal was not significantly affected by ionic strength. The electrical-double-layer compression and resulting zeta potential decrease did not

increase the removal of microalgae biomass. It was suggested that this could be related with the physiological ability of algae may allow the cells to counterbalance electrical neutralization as a result of the addition of a coagulant or other ions for flocculation.

5.2. FUTURE WORK

This work has highlighted the benefit and possibilities of *Chlorella* coagulation/ flocculation process. The level of removal achieved with pH adjustment and the overall downstream solid-liquid separation processes can be optimized. Research is required in this area and should concentrate on:

- Electrolytic flotation. Literature review highlighted this technology as one most promising. It consumes relatively little energy, it is easy to control and it results in an efficient separation of the algae (> 90%). Moreover, since it is not contaminated with toxic flocculants, the harvested algal biomass can afterwards be used for different purposes such as algal feed and food. Further research on electrolytic flocculation would have to look for application development at the pilot scale.

- Repeat the proceedings with the brown algae. This work has highlighted the relationship between zeta potential and algae life stages and the possibility of inducing coagulation and removal with pH control. However, the challenging attempt to induce the high oil stage was unsuccessful and this drawback did not allow a full characterization of all the life stages of the *Chlorella* strain produced.

- Extracellular organic matter (EOM) characterization in different life stages. It has been postulated the composition and quantity of excreted EOM controls the surface charge as opposed to the cell surface itself and EOM decreases with increasing population age. More research is required based for example on Scanning Electronic Microscope techniques in order to test the strength of the relationships identified.

- Characterization of the culture medium at the time of flocculation, namely testing calcium, magnesium, carbonate, and phosphate concentrations at the time that the jar tests would be performed in order to enhance the coagulation-flocculation rate and consequently biomass removal.

Chapter VI

6. REFERENCES

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